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Introgression of Botrytis grey mould resistance genes from *Cicer reticulatum* (*bgmr1*$_{cr}$) and *C. echinospermum* (*bgmr1*$_{ce}$) to chickpea (*C. arietinum*)

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Abstract

Botrytis grey mould (BGM), caused by the fungus *Botrytis cinerea* Pers. ex Fr., is an important disease of chickpea causing economic losses across the world in chickpea-growing regions. There are no available resistance sources in cultivated chickpea against this disease. *Cicer echinospermum* and *C. reticulatum*, the only two compatible annual wild species, have been reported to have resistance to BGM. Hence, interspecific populations were developed with susceptible cultivars as female parents and *C. echinospermum* accession IG 73074 and *C. reticulatum* accession IG 72937 as the pollen donors to transfer and assess the nature of genetic control for BGM. Screening the progeny indicated that resistance to BGM was controlled by a single additive gene/allele (*bgmr1*$_{cr}$ and *bgmr1*$_{ce}$), which can be introgressed through a backcross breeding programme.

Keywords: Botrytis-grey mould; chickpea; *Cicer arietinum*; *Cicer echinospermum*; *Cicer reticulatum*; disease resistance; interspecific cross

Introduction

Botrytis grey mould (BGM), a disease caused by the necrotrophic fungus *Botrytis cinerea* Pers. ex Fr., has been reported from more than 15 countries (Nene *et al.*, 1984). BGM is one of the most devastating diseases of chickpea (*Cicer arietinum* L.) and can result in complete yield loss (Davidson *et al.*, 2004). It was first reported in the Jujuy Province of Argentina, causing 95% crop loss (Carranza, 1965). It is a serious constraint to chickpea production in many Asian countries including northern India, Nepal, Bangladesh and Pakistan. The disease is considered to be the major cause for the decline in the chickpea-growing areas of Nepal and Bangladesh (Pande, 1998). More than 80% yield loss has been observed in chickpea crops grown on the Indo-Gangetic plains of India (Pande, 1998). High levels of resistance have not been found in the cultivated germplasm (Singh and Bhan, 1986), which has encouraged the search for resistance sources in the related wild species.

Evaluation of germplasm accessions of wild species has revealed that they possess a wealth of genes for biotic and abiotic stresses (Haware *et al.*, 1992, Mallikarjuna, 2003; Gaur *et al.*, 2009). They have resistance to three or more stresses such as Ascochyta blight, BGM and *Fusarium* wilt (Robertson *et al.*, 1995). *Cicer reticulatum* and *C. echinospermum*, two wild relatives from the secondary gene pool of chickpea (Mallikarjuna *et al.*, 2011), have been reported to be resistant to BGM (Singh *et al.*, 1991; Singh *et al.*, 1998; Ramgopal,

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2006). In addition, accessions of *Cicer bijugum*, *C. pinnatifidum* and *C. judaicum* from the tertiary gene pool are resistant, but these species are currently inaccessible for chickpea improvement due to incompatibility between these species and cultivated chickpea (Mallikarjuna *et al.*, 2011). Wild relatives in the secondary gene pool of chickpea are amenable to wide crossing and gene transfer (Collard *et al.*, 2003; Mallikarjuna *et al.*, 2011). Nevertheless, until now, none of the wild *Cicer* species have been used in the crossing programme to transfer BGM resistance to cultivated chickpea.

Currently, deployment of host plant resistance has limited potential in BGM management, as high levels of resistance have not been identified in cultivated germplasm and because of the variable nature of *B. cinerea* populations (Davidson *et al.*, 2004). Fungicidal control of BGM is expensive and development of fungicide resistance has been reported frequently in *B. cinerea* populations (Leroux, 2004). This study describes the introgression of BGM resistance from *C. reticulatum* and *C. echinospermum* into chickpea and the pattern of inheritance of resistance.

Materials and methods

Plant material

The experiment was conducted at the International Crops Research Institute for Semi Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. The Genetic Resources Unit of ICRISAT provided *C. reticulatum* (collected from Turkey) and *C. echinospermum* (collected from Turkey) seeds, which were multiplied and used in the crossing programme. The crossing programme was carried out using the BGM-resistant accessions of *C. echinospermum* IG 73 074 (ICC 20 192) and *C. reticulatum* IG 72 937 (ICC 20 170) as male parents. Chickpea cultivars ICC 4954 and ICC 92 318, both susceptible to BGM, were used as female parents to develop interspecific populations. The F_1s developed were selfed to develop F_2 and backcrossed to the cultivated chickpea parent to develop BC_1 populations in the glasshouse.

Screening for BGM

For the identification of BGM resistance, 8–10-d-old test seedlings along with the susceptible parent chickpea cultivars ICC 92 318 and ICC 4954 were inoculated with *B. cinerea* on a potato dextrose agar medium, which was isolated from naturally infected chickpea plants collected from the BGM hot-spot location Pantnagar, India. The isolate was from single spore following standard mycological procedures. Conidia of *B. cinerea* were cultured on autoclaved marigold (*Tagetes erecta*) flowers. The conidia were harvested into sterile distilled water, adjusted to 3×10^5 conidia/ml using a haemocytometer and used as an inoculum. Chickpea seedlings were sprayed with the inoculum using a hand-operated atomizer. The inoculum was allowed to partially dry for about 30 min. Inoculated plants were maintained at 15 ± 2°C and above 60% relative humidity with a 12 h photoperiod. Disease scores for BGM on each accession were recorded using a 1–9 rating scale at 20 d after inoculation. The disease rating from scores 1 to 3 was treated as resistant, scores 4 to 5 as moderately resistant and scores 6 to 9 as susceptible (Pande *et al.*, 2006).

Data analysis

A χ^2 goodness-of-fit test was calculated as given by Panse and Sukhatme (1967) and the calculated χ^2 values were compared with table values given by Fisher and Yates (1963), against appropriate degrees of freedom (df).

Results

In this study, two crosses, one derived from *C. reticulatum* (ICC 92 318 × IG 72 937) and another derived from *C. echinospermum* (ICC 4954 × IG 73 074), were developed to screen for BGM resistance. The disease symptoms in the susceptible cultivar began with wilting and decaying of the leaves followed by decaying of the aerial parts of the plant by 18–20 d post-inoculation (Fig. 1(b)). In the resistant *C. echinospermum* (Fig. 1(a)) and the resistant interspecific derivatives (Fig. 1(c)), the aerial parts remained green and fresh without any signs of wilting and decaying. Wilting and decaying of the aerial parts of the seedlings was observed in disease-susceptible interspecific derivatives (Fig. 1(d)). Plants that did not show any disease symptoms upon transplantation to suitable pots grew further and set flowers and pods. Susceptible plants continued to decay under disease pressure.

The F_2 population derived from the *C. reticulatum* IG 72 937 cross (ICC 92 318 × IG 72 937) had a total of 16 plants. BGM disease screening tests showed three plants to be resistant while nine plants were found to be moderately resistant and four were susceptible to the disease. The BGM disease reaction fitted into a 1 (resistant):2:(moderately resistant):1 (susceptible) monogenic segregation ratio with additive gene action (χ^2 test ratio 0.38^{ns}, $P = 0.83$ at 2 df). Similarly, the BC_1 population (derived by crossing the F_1 of the same cross to ICC 92 318) had a total of 20 plants in which eight plants were moderately resistant to the disease and 12 plants were susceptible.

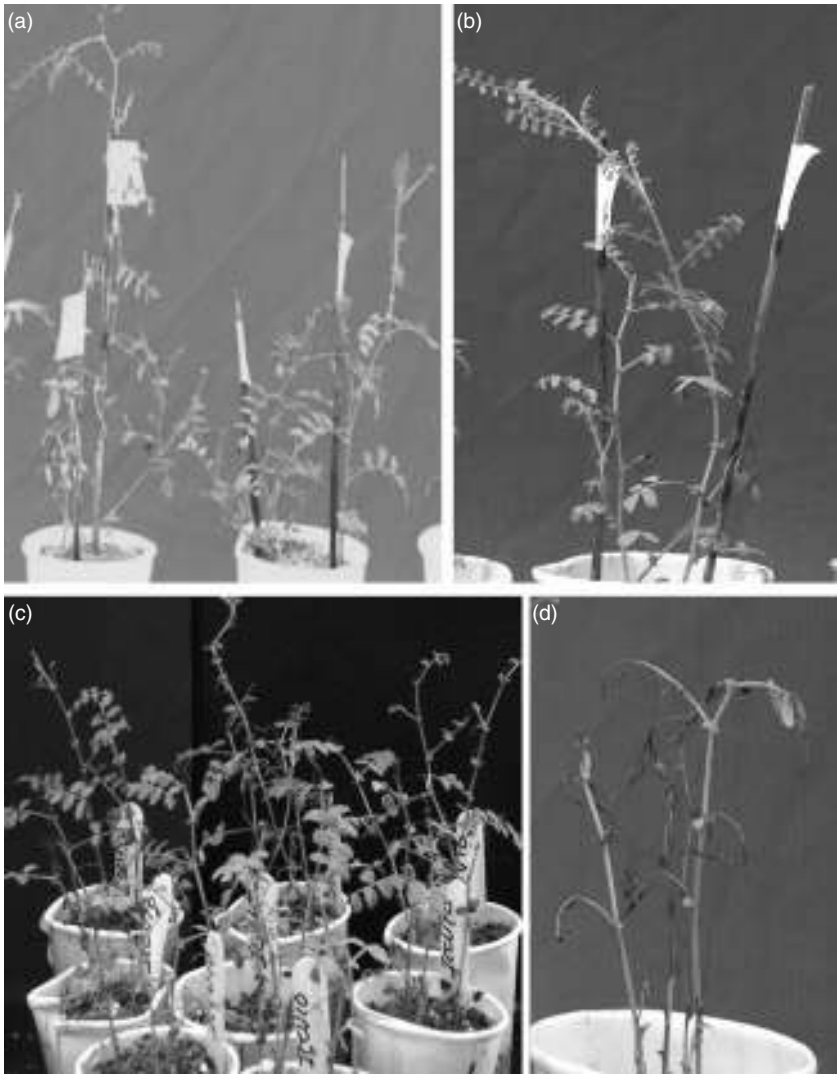

Fig. 1. Botrytis grey mould screening of interspecific derivatives between *C. arietinum* and *C. echinospermum*. (a) Wild species, *C. echinospermum,* showing no BGM disease. (b) Cultivated chickpea cultivar showing susceptibility to the disease. (c) Interspecific derivative resistant to the disease. (d) Interspecific derivative showing susceptible reaction to the disease (a colour version of this figure can be found online at journals.cambridge.org/pgr).

The χ^2 test (0.8^{ns}) with a P value of 0.37 at 1 df followed the ratio 1:1, thereby confirming the monogenic additive F_2 segregation ratio. The progeny derived from C. reticulatum were advanced further and screened for BGM, and the lines showed resistance to BGM.

The F_2 population derived from the *C. echinospermum* IG 73074 cross (ICC 4954 × IG 73074) had a total of 63 plants, of which 12 plants were resistant, 37 moderately resistant and 14 susceptible to the disease. The χ^2 test was carried out to test whether the ratio of 1:2:1 fitted. The χ^2 test was 2.05^{ns} with a P value of 0.36 at 2 df. The BC_1 population (obtained by crossing the F_1 of the same cross to ICC 4954) had a total of 17 plants with seven moderately resistant plants and 10 susceptible plants. The χ^2 test (0.53, n.s.) with a P value of 0.47 at 1 df confirmed the ratio of 1:1 for the backcross. These results indicate that BGM resistance inherited from both *C. reticulatum* and *C. echinospermum* was a single gene showing additivity.

Discussion

This is the first report on the introgression of BGM resistance from wild relatives of *Cicer*, namely *C. reticulatum* and *C. echinospermum*, into *C. arietinum*. In the present study, single and additive modes of resistance were observed for BGM. The additive alleles from *C. reticulatum* (designated as $bgmr1_{cr}$) and *C. echinospermum* (designated as $bgmr1_{ce}$) were needed in two

doses ($bgmr1_{cr}$/$bgmr1_{cr}$; $bgmr1_{ce}$/$bgmr1_{ce}$) to impart complete resistance, while heterozygous ($Bgmr1_{cr}$/$bgmr1_{cr}$; $Bgmr1_{ce}$/$bgmr1_{ce}$) individuals were moderately resistant. Disease resistance genes present in the wild species are recessive in many crop plants, as seen in *Cajanus platycarpus*, where resistance to *Phytophthora* blight has been reported to be monogenic and recessive (Mallikarjuna *et al.*, 2005). Collard *et al.* (2003) reported digenic and recessive modes of resistance to Ascochyta blight in the interspecific derivatives of chickpea from *C. echinospermum* accession ICC 17 159. The results from the present study indicated that neither susceptibility nor resistance was dominant over the other. Previous reports on the transfer of BGM resistance from cultivated chickpea lines showed single dominant gene, and between two resistant cultivated chickpea parents showed duplicate dominant genes (Singh, 1997). The nature of resistance was probably moderate to low as the plants succumbed to the disease when the infection was moderate to severe. Some more examples of *Cicer* species contributions to chickpea improvement are successful introgression of Phytophthora root rot resistance from *C. echinospermum* (Knights *et al.*, 2008) and introgression of nematode resistance from *C. reticulatum* and *C. echinospermum* (Gaur *et al.*, 2009). *Cicer pinnatifidum*, *C. judaicum* and *C. bijugum* are known to possess resistance to *Fusarium* wilt, Ascochyta blight, BGM and bruchids (Stevenson and Veitch, 1998; Mallikarjuna *et al.*, 2011).

These results indicate that when desired levels of resistance to biotic constraints are lacking in the cultivated or primary gene pool, there is an option for sources of resistance in the secondary gene pool where the species are cross-compatible and offer genetic variability to tackle many of the biotic constraints (van der Maesen *et al.*, 2007; Mallikarjuna *et al.*, 2011). It was possible to transfer *Helicoverpa armigera* resistance from *C. reticulatum* (Mallikarjuna *et al.*, 2007, Mallikarjuna *et al.*, 2011). Therefore, as demonstrated in the present study, *C. reticulatum* and *C. echinospermum* with their valuable sources of variation for BGM resistance offer genetic variability to broaden the genetic base of cultivated chickpea and introduce useful traits not present in the cultivated gene pool.

Further studies on allelism are needed to ascertain whether the two BGM resistance genes ($bgmr1_{cr}$ and $bgmr1_{ce}$) reported in this study are allelic variants of the same gene, or are two different genes.

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